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Exposures to molds in school classrooms of children with asthma

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Abstract

Background: Students spend a large portion of their day in classrooms which may be a source of mold exposure. We examined the diversity and concentrations of molds in inner-city schools and described differences between classrooms within the same school.

Methods: Classroom airborne mold spores, collected over a 2 day period, were measured twice during the school year by direct microscopy.

Results: There were 180 classroom air samples collected from 12 schools. Mold was present in 100% of classrooms. Classrooms within the same school had differing mold levels and mold diversity scores. The total mold per classroom was 176.6 ± 4.2 spores/m3 (geometric mean ± standard deviation) and ranged from 11.2 to 16,288.5 spores/m3. Mold diversity scores for classroom samples ranged from 1 to 19 (7.7 ± 3.5). The classroom accounted for the majority of variance (62%) in the total mold count, and for the majority of variance (56%) in the mold diversity score versus the school. The species with the highest concentrations and found most commonly included Cladosporium (29.3 ± 4.2 spores/m3), Penicillium/Aspergillus (15.0 ± 5.4 spores/m3), smut spores (12.6 ± 4.0 spores/m3), and basidiospores (6.6 ± 7.1 spores/m3).

Conclusions: Our study found that the school is a source of mold exposure, but particularly the classroom microenvironment varies in quantity of spores and species among classrooms within the same school. We also verified that visible mold may be a predictor for higher mold spore counts. Further studies are needed to determine the clinical significance of mold exposure relative to asthma morbidity in sensitized and non-sensitized asthmatic children.

Mold spores are ubiquitous in the indoor and outdoor environment. They play an important role in nature through the recycling of organic matter into useful nutrients. Mold spores are also responsible for a number of health-related diseases as they can be allergens, irritants, infectious agents, or produce toxins. Several studies have suggested that mold sensitization is associated with asthma development and asthma morbidity (1–10). Additionally, a smaller literature links exposure to elevated mold pathogens such as mycotoxins or microbial volatile organic compounds (MVOC) to wheezing, development of asthma and increased asthma morbidity in non-sensitized children with asthma (11–17).

The majority of indoor studies of mold have focused on the home environment or on 'sick buildings' (e.g., the work environment). Few have provided detailed assessments of school or classroom exposures; however, the school/classroom environment potentially plays an important role in mold exposure, as children spend a large portion of their day in school. We measured the concentrations of airborne molds during two seasons inside 12 inner-city elementary schools in

the Northeast United States. The objective of this study was to examine the diversity, concentrations, and the presence of molds in these schools; to describe the differences between schools and classrooms; and to evaluate seasonal trends and predictors of total mold levels.

Methods

The National Institute of Allergy and Infectious Diseases funded School Inner-City Asthma Study (SICAS) (R01AI073 964) is an ongoing longitudinal study whose primary purpose is to evaluate the role of indoor allergens specific to the inner-city classroom environment and asthma morbidity in 400 students from 40 inner-city elementary schools. Recruitment is ongoing. The study design has been previously reported (18). Briefly, children with physician-diagnosed asthma attending inner-city schools were recruited from screening surveys collected during the spring and phenotypically characterized at baseline in the summer. The enrolled students were then followed longitudinally for asthma morbidity outcomes during the subsequent academic school year, while school and home environmental exposure assessments were made. With permission from the school superintendent, settled classroom dust and air sampling for environmental allergens were collected twice during the academic school year and linked to the enrolled students with asthma. Only classrooms of asthmatic children who were part of this study were sampled. Every year, a unique group of students are recruited from 5 to 7 different urban, elementary schools. The study was approved by the institutional review board of Boston Children's Hospital and the Brigham and Women's Hospital.

Mold air sampling

Airborne mold spores were collected in each classroom using Burkard Indoor Recording Air Samplers (Burkard Mfg. Co., Rickmansworth, Herts, UK). Samplers were placed on the floor, in the periphery of the room, and away from entryways or operable windows.

Slides were microscopically analyzed at 1000× magnification. A segment of the slide representing the school day (8:00 am until 4:00 pm) was marked, a portion of which was scanned, and all mold spores encountered were identified and counted. Raw counts were converted to airborne concentrations using the sampler flow rate, exposure time, and percent of the collection surface analyzed. Results were reported as spores per cubic meter of air (spores/m^3) for the 8-h collection period (18, 19). Two consecutive 8-h collection days were averaged for each classroom.

Mold analysis

A 'total mold' category was calculated as the sum of all mold groupings. Individual mold groupings were reported, and 'unidentifiable' was used to categorize spores that were not morphologically identifiable but also a few rarely encountered types that did not fit into the following groupings. *Penicillium* and *Aspergillus* were reported together as they are usually too similar morphologically to differentiate by direct microscopy. Although basidiospores are discussed as a group in the results, to categorize mold groups and determine mold diversity scores, they were separated into four categories: small hyaline basidiospores, *Coprinus*, *Ganoderma,* and other basidiospores. Likewise, ascospores were separated into *Chaetomium*, *Leptosphaeria*, *Xylariaceae*, *Diatrype*-like and *Paraphaeosphaeria michotii*, and 'other ascospores'. Hyphal fragments were not included in the 'total fungus' calculation but were reported as a separate fungal category.

Seasonal analysis

As previously described, classrooms were sampled twice annually, in the Fall and Spring, during the academic school year with approximately 6 months between sample collections (18).

Mold diversity

For assessment of mold diversity, a score was generated for each classroom sample by summing the number of mold groupings present (excluding total mold, hyphae and unidentifiable spores). This score could range from 1 to 28. A score of 1 indicated only one mold grouping detected, where as a score >1 indicated multiple mold groupings detected.

Home and classroom environment dust sampling

The major scope and funding for this study were focused on the school environment. We did not have airborne mold spore sampling in the students' homes. To try to account for the home exposure, we did a small analysis of home and school dust samples. *Alternaria alternata 1* (Alt a 1) was measured by Luminex microarray™ (Indoor Biotechnologies, Charlottesville, VA, USA) from vacuum dust samples collected from each SICAS students' home (bedroom) and classroom. The lower limit of detection was 0.004 μg/g. Evaluation of other mold species by vacuum dust was beyond the scope of this study, given that extensive mold air sampling was already being performed in the classrooms, and due to the known limitations of dust sample analysis for molds (20).

Classroom and home survey for detection of mildew/dampness

Dampness was evaluated in the classroom and the home. Study staff evaluated classroom dampness in the Fall and Spring by observing for the presence of mildew or water stains on the ceiling, walls, or window. Mildew was defined as visible mold. Parental survey ascertained whether there was mildew present in the home in the past 12 months.

Statistical analysis

Analyses are based on the first 2 year of study data. Geometric means were calculated for each mold grouping and total mold. To account for a high frequency of zero values, as was the case where certain fungal groups were rarely recovered, a value of 1

spore/m^3 was added to all concentrations and then subtracted from the calculated geometric mean (21). Mold levels were compared between rooms with and without mildew using generalized estimating equations (GEE) to account for the non-independence of two samples (i.e., Spring and Fall) coming from the same classroom. Comparisons of mold by season were made using paired t-tests. Mixed-effects linear regressions were used to determine the variance in molds that was attributable to the school and classroom levels. Comparisons for Alt a 1 were made using Fisher's exact test (Fall only) and comparisons for dampness were made using GEE (Fall and Spring samples). Analyses were generated using STATA 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP.).

Results

There were 180 classroom air samples collected from 12 schools (five schools in year 1 and seven schools in year 2). Ninety-two air samples were collected from the Fall and 88 air samples from the Spring. One hundred and seventeen vacuum dust samples were collected from the SICAS students' bedrooms. One hundred and ninety-five vacuum dust samples were collected from the schools.

School and classroom variation

Table 1 reviews the demographics of the 12 schools including year of construction, number of classrooms sampled, and geometric means of the total mold concentration per school. The year of construction ranged from 1904 to 1975. There was no relationship between total mold concentration and school age. There was wide variation in total mold spore concentrations across the 12 schools. The geometric mean ranged from 32.5 spores/m^3 in school 6 to 545 spores/m^3 in school 7.

The number of samples collected per school ranged from 2 to 34 classroom samples. Fig. 1 illustrates the wide variability in total mold quantity by classroom within each school. A mixed-effects model, which used season as a fixed effect, determined that the school level accounted for 38% of the variance in total mold quantity, leaving 62% of the variance attributable to the classroom level, thus demonstrating substantial variability between classrooms within the same school.

Table 1 School characteristics and mold spore levels

School ID	Year built	Classrooms sampled	Total mold Geomean ± Geostd (spores/m^3)	Min	Max
1	1972	34	217.8 ± 2.7	22.5	2430.6
2	1922	13	114.6 ± 1.9	42.4	342.8
3	1959	33	498.1 ± 6.0	44.7	16,288.5
4	1904	12	332.4 ± 3.6	85.8	8762.7
5	1925	9	493.0 ± 1.5	281.5	996.1
6	1975	24	32.5 ± 1.7	11.2	98.7
7	1932	7	545.0 ± 3.4	132.4	5025.6
8	1924	2	158.6 ± 2.1	93.5	269.2
9	1905	13	131.1 ± 1.4	56.1	179.5
10	1963	16	72.9 ± 2.4	19.0	544.1
11	1972	4	319.0 ± 3.4	82.3	919.8
12	1975	13	130.0 ± 4.0	29.9	7067.3

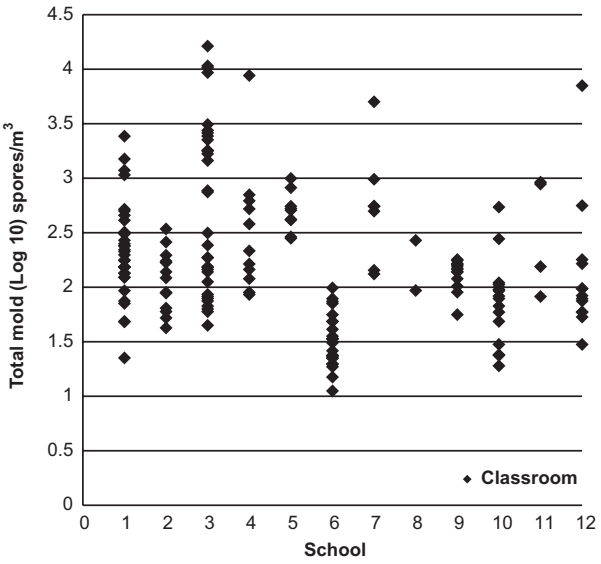

Figure 1 There is substantial variability in total mold quantity between classrooms within the same school. The values shown are the log 10 of the geometric means for total mold for each classroom.

Distribution of molds

The prevalence, quantity, and range of 28 different mold groupings are summarized in Table 2. Mold was present in all classrooms (180 of 180). The most prevalent identifiable spores were *Cladosporium*, basidiospores, *Penicillium*/*Aspergillus,* and smut spores.

The geometric mean of the total mold was 176.6 ± 4.2 spores/m^3 ranging from 11.2 to 16,288.5 spores/m^3 respectively. Of the identifiable spores, *Cladosporium* had the highest geometric mean of 29.3 ± 4.2 spores/m^3. Also present in abundant quantities were basidiospores, *Penicillium*/*Aspergillus*, and smut spores.

Regarding the traditional 'indoor molds' (those associated with dampness/moisture damage), *Penicillium*/*Aspergillus* was the most prevalent and found at the highest concentration (detected in 88% of samples, geometric mean of 15.0 ± 5.4 spores/m^3). *Bispora* was detected in one-third of the classrooms. *Chaetomium* was infrequently recovered (9% of classrooms), *and Scopulariopsis* and *Stachybotrys* were rarely (4% of classrooms) recovered.

We defined a mold diversity score as the total number of mold types present in a classroom. The mold diversity score in the 180 classroom samples ranged from 1 to 19. On average, classrooms had approximately seven mold groupings present

Table 2 Distribution of molds in classrooms (n = 180)

Mold grouping	Geomean ± Geostd (spores/m^3)	Detectable* (%)	Min	Max	Percentiles 25th	50th	75th
Total mold	176.6 ± 4.2	100	11.2	16,288.5	73.3	145.9	388.5
Hyphae	55.2 ± 2.8	98	0	555.4	33.7	59.9	92.4
Unidentifiable spores	31.2 ± 2.8	98	0	448.8	19.2	33.6	56.1
Cladosporium	29.3 ± 4.2	97	0	1525.7	11.7	28.9	71.4
Smut spores (Ustilaginomycetes)	12.6 ± 4.0	89	0	639.4	7.5	15.0	34.0
Penicillium/Aspergillus	15.0 ± 5.4	88	0	8586.0	4.0	15.0	46.7
Other Basidiospores†	6.6 ± 7.1	67	0	2445.5	0.0	7.5	22.5
Basidiospores small hyaline†	4.9 ± 9.6	54	0	11,173.1	0.0	3.8	11.3
Other Ascospores	2.7 ± 5.5	48	0	956.5	0.0	0.0	7.5
Bispora	1.1 ± 3.1	35	0	127.0	0.0	0.0	3.8
Coprinus†	1.2 ± 3.6	32	0	149.6	0.0	0.0	3.8
Myxomycetes	0.7 ± 2.5	31	0	74.8	0.0	0.0	3.8
Rust spores (Pucciniomycetes)	0.7 ± 2.4	31	0	29.8	0.0	0.0	3.8
Alternaria	0.7 ± 2.5	29	0	37.4	0.0	0.0	3.8
Ganoderma†	0.9 ± 3.4	28	0	403.9	0.0	0.0	3.8
Other Mitospores	0.5 ± 2.4	21	0	740.2	0.0	0.0	0.0
Leptosphaeria‡	0.4 ± 2.2	17	0	53.6	0.0	0.0	0.0
Curvularia	0.4 ± 2.2	16	0	52.2	0.0	0.0	0.0
Bipolaris/Drechslera-like	0.3 ± 1.9	13	0	29.9	0.0	0.0	0.0
Xylariaceae‡	0.2 ± 1.9	12	0	59.9	0.0	0.0	0.0
Pithomyces chartarum	0.2 ± 1.8	12	0	15.0	0.0	0.0	0.0
Chaetomium‡	0.2 ± 1.6	9	0	29.9	0.0	0.0	0.0
Epicoccum nigrum	0.2 ± 1.6	9	0	15.4	0.0	0.0	0.0
Botrytis	0.2 ± 1.8	8	0	30.0	0.0	0.0	0.0
Diatrype-like‡	0.2 ± 2.0	8	0	82.3	0.0	0.0	0.0
Stachybotrys	0.1 ± 1.5	4	0	15.4	0.0	0.0	0.0
Myrothecium	0.1 ± 1.6	4	0	29.9	0.0	0.0	0.0
Scopulariopsis	0.1 ± 1.6	4	0	55.2	0.0	0.0	0.0
Torula	0.03 ± 1.3	2	0	15.0	0.0	0.0	0.0
Paraphaeosphaeria michotii‡	0.03 ± 1.2	2	0	7.5	0.0	0.0	0.0
Periconia-like	0.02 ± 1.2	1	0	7.5	0.0	0.0	0.0

*Detectable is defined as percentage of classrooms where a particular fungal type was identified at least once.
†Basidiospore.
‡Ascospore.

(mean = 7.7, 25–75% of 5.0–10.0). A mixed-effects model demonstrated that the school level accounted for 44% of the variance in the number of mold groupings per sample, leaving 56% of the variance attributable to the classroom level, thus confirming substantial between classroom differences in mold diversity within the same school.

Classrooms with mildew

Table 3 reports the difference in mold spore concentrations in classrooms with mildew present vs. those without. 27 (15%) classrooms were reported to have mildew on the ceiling, wall, or window. Total mold spores and the most prevalent mold groupings were compared between classrooms. The means were consistently higher for rooms with mildew present and significantly higher for total mold, *Cladosporium,* basidiospores, and *Alternaria*.

Seasonal variation

Mold concentrations varied by season. This was estimated by comparing classrooms that had both a Fall and Spring sample (n = 80 for each season). Total mold and the most predominant mold groupings were compared and were significantly higher in the Fall as shown in Table 4.

Home and classroom vacuum dust samples and dampness assessments

Assessments were made in the Spring and Fall. In the Spring, there were no positive dust samples for Alt a 1. In the Fall, Alt a 1 was detectable in more classroom samples, 9/100 (9%), as compared to home samples, 0/81 (p = 0.005). Significantly more classrooms 27/180 (15%) reported signs of mildew as compared to homes, 4/138 (2.9%) (p = 0.001).

Table 3 Comparison of molds in classroom with mildew† visualized vs. not visualized

	Mildew visualized in rooms (n = 27)		Mildew NOT visualized in rooms (n = 139)		
Mold Grouping	Geomean ± Geostd (spores/m^3)	Detectable (%)	Geomean ± Geostd (spores/m^3)	Detectable (%)	p-value*
Total mold	305.6 ± 6.0	100	163.5 ± 3.9	100	0.03
Cladosporium	54.7 ± 5.2	100	26.4 ± 4.0	96	0.02
Penicillium/Aspergillus	19.0 ± 4.9	88	15.1 ± 5.3	89	0.56
Other basidiospores	23.7 ± 12.7	81	5.3 ± 6.0	65	0.001
Basidiospores small hyaline	19.0 ± 18.2	77	4.0 ± 8.0	52	0.003
Smut spores	15.8 ± 3.8	89	11.7 ± 4.1	88	0.37
Alternaria	1.6 ± 3.3	46	0.6 ± 2.4	26	0.02
Stachybotrys	0.2 ± 1.8	12	0.1 ± 1.4	4	0.12

*Two-sample t-test of log-transformed mold concentrations.
†Mildew is defined as the presence of visible mold.

Table 4 Comparison of molds by season

	Fall (n = 80)		Spring (n = 80)		
Mold grouping	Geomean ± Geostd (spores/m^3)	Detectable (%)	Geomean ± Geostd (spores/m^3)	Detectable (%)	p-value*
Total mold	252.0 ± 5.6	100	121.0 ± 3.0	100	0.004
Cladosporium	48.4 ± 5.0	98	17.2 ± 3.2	95	<0.001
Penicillium/Aspergillus	22.0 ± 5.6	91	9.9 ± 5.4	83	0.002
Other basidiospores	13.2 ± 11.2	68	2.9 ± 3.3	65	<0.001
Basidiospores small hyaline	7.6 ± 14.5	54	3.1 ± 5.9	53	0.004
Smut spores	16.1 ± 5.0	86	9.5 ± 3.0	91	0.02

*Paired t-test of log-transformed mold concentrations.

Discussion

Few published studies provide a comprehensive evaluation of airborne mold spores in classrooms (22–25). Our study, with a focus on the classrooms of asthmatic children from urban Northeast United States schools, is one of the largest to do so. We demonstrated that mold was found in all classrooms, but there was a wide range in the quantity and diversity of mold present. Interestingly, classrooms within the same school had different mold levels and different mold diversity scores. Our data supports high and variable levels of classroom-specific mold exposure, even within schools, suggesting classroom-specific exposures may be important sources of allergen or irritant mold exposure.

There was a high degree of variance in the quantity and diversity of molds between classrooms in the same school. School 3 demonstrated that the total mold spore count varied from 44.7 spores/m^3 in one classroom to 16,288.5 spores/m^3 in another. The classroom accounted for the majority of variance (62%) in the total mold count and accounted for the majority of variance (56%) for the mold diversity score. These findings emphasize the importance of assessing the microenvironment. Classroom location, room shadiness vs. sunlight, water incursions, high indoor humidity, and temperature may all be contributing factors. The frequency for which windows are opened or structural abnormalities in the school and classroom may allow easier penetration of the outdoor air spores. The variability of these data underscore the importance of obtaining samples from multiple classrooms, because exposure seems to be classroom specific and obtaining a single school sample can potentially misclassify the exposure.

The predominant mold types recovered were *Cladosporium, Aspergillus/Penicillium*, and basidiospores, which are species generally known to cause symptoms in sensitized individuals. These findings are consistent with other studies that sampled indoor environments (22, 23, 26–29). *Penicillium/Aspergillus* were the most prevalent of the traditional 'indoor' molds, found most consistently (in 88% of classrooms) and at the highest concentrations. In contrast, *Alternaria* was found in just 29% of rooms and not in high concentrations. Other molds that are commonly associated with indoor water damage or decay such as *Stachybotrys, Chaetomium, Scopulariopsis, and Bispora* were found in low concentrations or were rarely recovered. Our data also showed a high concentration of hyphal fragments. Hyphae are filamentous, typically non-reproductive parts of the fungus that can share common antigens with spores (30). High concentrations of airborne hyphae can be an indication of unusually high fungal growth. Several studies have shown that these small fungal particles contribute to respiratory disease (31–33).

Allergy testing is routinely performed to check for sensitization to common molds. While some of the species found in the classrooms (*Cladosporium, Aspergillus*/*Penicillium*) are considered well known to be clinically important in sensitized individuals, some of the other less common classroom mold species for which there are no reliable or specific allergy tests may also have health effects. Although mold skin test extracts are largely non-standardized, it is possible that testing to other molds may help identify sensitized children who have increased asthma or rhinitis symptoms while at school.

We found that visualized mildew was a predictor of increased mold spore levels. As seen in Table 3, classrooms with visualized mildew had significantly higher levels of many of the common molds. The presence of mold spores indoors can either result from indoor moisture issues and/or from outdoor molds that have gained indoor access (34). *Cladosporium*, *Alternaria,* and basidiospores are nearly ubiquitous in the outdoor air and often penetrate indoors. Some of these molds can proliferate further indoors under appropriate conditions. Additionally, leaking roofs, faulty plumbing, poor drainage, or wet carpets may lead to focal areas of mold growth. However, it is well acknowledged that there is no gold standard for measuring mold exposure and that visualization and measurements in the air and dust are complementary, but not necessarily tightly correlated (27).

There was a seasonal relationship as the mold spore concentrations were higher earlier in the academic school year. Ambient spore counts reach a peak in the late Summer and Fall months and subsequently decrease with the frost (35). Our study had similar findings with increased spores during the Fall season when outdoor molds are often at a peak concentration in the Northeast United States. During these months, there is a significant increase in decaying vegetation.

Some factors were not predictive of increased mold levels. School age did not correlate with total mold as mold was found in both new and older schools. Issues such as plumbing problems, leaky roof, malfunction in the ventilation system, or poor structural integrity could be factors that are more likely affected by maintenance resources rather than school age.

A strength of this study is that our sampling methods utilized continuous air sampling through the entire school day, which may reflect a more accurate exposure over long periods of time by averaging the short-term spore concentration peaks and troughs typically observed. We also used direct microscopy for identification and quantification of mold spores because it gave us the opportunity to report the most spore types, regardless of viability or culturability, and may give a measure of more clinically relevant molds than other methods.

We were limited in our ability to compare school vs. home exposures to mold. We only measured airborne mold spores in the classrooms and not in the homes, but we do have measures of Alt a 1 from settled dust in both settings and, we also ascertained reports of home dampness in the classrooms and the homes. Alt a 1 is not a highly prevalent mold indoors, but we did detect some Alt a 1 in classroom dust, whereas we detected no Alt a 1 in the homes of our participants. Dampness, a condition that can result in mold growth, was also reported more commonly in the classrooms compared with the homes. We have seen that urban housing in the Northeast United States, specifically apartment housing, has heating that may be high in the winter. Warm dry air likely results in the low levels of dust mites we have measured previously in homes, and perhaps in the decreased dampness and absence of Alt a 1 that was measured in homes in this study (36). In contrast, the urban classroom/school environment may be more likely to support the growth of a number of different molds and may be an important source of exposure for urban school children with asthma. Several studies have found that exposure to dampness or visible mold can be associated with wheezing or asthma development (11, 13, 14). The mechanism for this effects is not well defined.

Our study has limitations. First, the sampling resources were directed only at the primary classrooms of enrolled SICAS students. As such, some schools had a small number of classrooms sampled. However, we believe that the breadth of the sampling across many classrooms and several schools outweigh the limitations posed by having some schools potentially under-sampled. No outdoor sampling was available for comparison to further characterize sources of molds and predictive factors, thus our ability to directly relate indoor to outdoor mold concentrations is limited. However, our sampling was performed during the academic school year in the Northeast United States, where conditions generally comprise of closed windows and limited exposure to outdoor molds. While some of the molds may have been carried in from outdoor sources, some of our highest concentrations included those molds more classically known as important in indoor environments, suggesting that there are likely direct indoor sources of mold in these classrooms.

Conclusion

This study demonstrates that the school/classroom environment can be a source of mold exposure both in quantity of spores and variety of mold types. In particular, we found that the classroom microenvironment varies among classrooms within the same school and that a classroom-specific mold sampling may provide the most accurate exposure data. Our study also verified an intuitive belief that the presence of visible mold may be a predictor for high mold spore counts. Further studies are needed to determine the clinical significance of mold exposure relative to asthma morbidity in sensitized and non-sensitized asthmatic children.

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